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14. ABSTRACT

The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. A translational research approach will provide the ability to find novel disease markers, optimal prevention and holistic treatment approaches, and a unique venue for future research as the "virtual laboratory" for optimal comprehensive health prevention in the military beneficiary population. This research method also allow us to further hypothesize and define relationships between CVD, other cardio metabolic disease states and maladaptive behavior patterns unique to service members such as pre-diabetes, stress, overweight and sleep disorders with the aim of targeting these disorders in a pre-clinical phase. Using an integrative, interdisciplinary preventive health approach, ICHP has shown that an individual's cluster of CV risk factors can be effectively targeted and improved.

15. SUBJECT TERMS
Lifestyle; Cardiac Prevention; Behavior; Coronary Heart Disease (CHD); Proteomics; Genomics; Metabolic Syndrome; Cardiovascular Disease (CVD); Diabetes; Obesity; Stress

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Annual Report

The Integrative Cardiac Health Project (ICHP) aims to lead the way in Cardiovascular Disease (CVD) Prevention by conducting novel research utilizing a Systems Biology / personalized medicine design to discover and develop practical, effective and preemptive integrative approaches in order to detect and combat CVD earlier before it affects the quality of life. ICHP's ultimate goal is to translate our evidenced-based research findings for application into clinical practice. In keeping with this aim, collaborative research efforts have continued between ICHP projects at Walter Reed Army Medical Center, Windber Research Institute and Windber Medical Center. In the year, the following key accomplishments are noted:

ICHP science has been transferred to Award No: W81XWH-11-2-0227

FY10 COE - (12 month NCE) - The remaining balance covers database development and minor equipment and supply purchases delayed due to the transition to WRNNMC. ICHP database platform creation successfully continues. Project anticipated completion date: December 2012.

FY09 CSI - (12 month NCE) - The available and committed amounts cover the collaborative research efforts between ICHP and Windber Research Institute (molecular studies). The following scientific updates are those of the collaborative research efforts between ICHP and Windber Research Institute.

1. Total annual visits:

CRC:
Annual visits (Aug 2011-Aug 2012) = 2,298 (last quarter June-Aug 2012 = *514*)

STEP:
Annual visits (Aug 2011-Aug 2012) = 160 (last quarter June-Aug 2012 = *25*)

2. Protocols completed:

Task #7: "Acute Endothelial Dependent Responses to Distinct Macronutrient Challenges Study: A Comparison of Brachial Reactivity Responses to a Low Carbohydrate/High Fat, High Carbohydrate/Low Fat, or an AHA Meal in Subjects at Risk for Coronary Heart Disease"

The following protocols are closed to enrollment, but remain open for data analysis:
Task #5: "Dean Ornish Program for Reversing Heart Disease"
Task #6: "Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal" and
"Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal: Sub-Study for Subjects in the Dr. Dean Ornish Program"

3. Active protocols:

Task #11: "Cardiovascular Risk Assessment and Prevention Program
Task #11a: Continue "Stress Therapy Empowering Prevention (STEP)" component to the Cardiovascular Risk Assessment program

Task #12:"Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Young Military Service Members"
Task #15: "Metabolic and Molecular Biology Studies in Surgical Interventions for Morbid Obesity"

Introduction

The epidemics of cardiovascular disease (CVD), Type II diabetes, and obesity generate a major share of the preventable costs of American health care. Currently, the American health care market place does not support preventive care that would save lives and costs associated with these problems. Healthcare costs are predicted to rise from 16% of the US GDP in 2005 to 30% of the GDP by 2025 if we fail to invest in prevention. The primary mission of the Integrative Cardiac Health Project (ICHP***),*** a congressionally-supported military-civilian collaboration between Walter Reed Army Medical Center (WRAMC) and Windber Medical Center (WMC)/Windber Research Institute (WRI) is to: 1) Teach, implement and study lifestyle changes added to "best" medical practices that promote cardiovascular health; 2) Identify patients at risk earlier by characterizing CVD even at the "molecular" disease stage and identifying biomarkers predictive of subclinical CVD; and 3) Relate genomic/proteomic changes to the evolution of CVD risk factors in response to lifestyle changes in an effort to prevent, arrest or reverse CVD. Within these objectives, ICHP will include: a) a comprehensive and innovative CVD risk factor assessment and prevention program in the military beneficiary population; b) advanced imaging methods for quantifying numerous aspects of heart health in military and other populations; c) an optimal healing environment for CVD patients; and d) an integrated statistical analysis of clinical, biochemical, and molecular data to identify patterns of CVD risk factors that will allow a unique and intensive collection of data at the clinical, biochemical, and molecular levels for heart disease, but with applicability and relevance in patients with other chronic diseases such as cancer, diabetes, metabolic syndrome and obesity. The heart disease data base will provide the ability to find novel disease markers, new or emerging evaluation and treatment approaches, and provide a unique venue for future research.

Body

Task #5: Transition away from the traditional Dr. Dean Ornish Program for Reversing Heart Disease protocol.

Status: Enrollment into the Dr. Dean Ornish Program is closed and all active participants have completed their participation in the study. Data analysis is ongoing.

Subject Enrollment and Demographics

This program is closed to enrollment and all active subjects have completed the program. Subject enrollment was 422 participants including 25 cohorts and 4 retreats. 339 participants graduated from the program and 83 participants discontinued participation (20% dropout rate). Demographic characteristics of participants were: average age of 66.1 years, 53% female, 33% veterans or the spouse of a veteran, and 41% had diagnosed coronary heart disease.

Outcome Data

Participants in the Dr. Dean Ornish Program at Windber Medical Center achieved significant improvement in levels of virtually all of the measured coronary artery disease (CAD) risk factors over the initial 12-week period. Measures of obesity including weight

and BMI declined ~7%, levels of total cholesterol were reduced by nearly 13%, blood pressure dropped ~9%, measures of physical fitness increased more than 26%, and levels of depression decreased approximately 47%. These data demonstrate that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease. Over the course of one year, weight and BMI decreased ~9%, diastolic blood pressure decreased ~7%, measures of physical fitness increased 25%, and levels of depression decreased nearly 50%.

Task #6: Complete enrollment in Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Subjects in the Dr. Dean Ornish Program protocol.

Status:
Enrollment to the global profiling study is closed and all active participants have completed their participation in the study. Enrollment in the sub-study was closed as of July 27, 2007. Data analysis is ongoing.

Subject Enrollment and Demographics
Subject enrollment was 374. There were 166 participants taking part in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study. Demographic characteristics of the control group were: average age of 63.7 years, 51% were female, 29% were veterans or the spouse of a veteran, and 34% had diagnosed coronary heart disease.

Data:

Inflammation biomarker panel – Findings reported previously.
Manuscript:
- Voeghtly LM, Neatrour DM, Decewicz DJ, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. Improvement in cardiometabolic risk factors during an intensive cardiovascular lifestyle intervention. Nutr Metab Cardiovasc Dis 2012 (*in press*).

Macrophage migration inhibitory factor (MIF) – MIF is an inflammatory cytokine that regulates smooth muscle cell migration and proliferation, and thus plays an important role in promoting development of atherosclerotic lesions. MIF has been shown to be an important biomarker for diseases with inflammation, such as CVD, diabetes, obesity, and cancer.

Genotyping of genetic variants in MIF gene that influence circulating levels completed; data analysis complete. MIF levels decreased significantly ($p<0.05$) in Ornish participants compared to controls at 12 weeks, but no difference in MIF levels between cases and controls at one year. Only women participants showed significant ($p<0.05$) reductions in MIF levels at 12 weeks (-23%). No change in men. Transcription of the human MIF gene is regulated by genetic polymorphisms in the MIF promoter, including the –173G/C single-nucleotide polymorphism and a sequence of tetra-nucleotide repeats at –794 (-794$CATT_{5-8}$). These polymorphisms may have relevance to cardiovascular disease, and this area has become a growing area of investigation; however, the tetranucleotide polymorphism and SNP variants were too infrequent for meaningful analysis.

Macrophage migration inhibitory factor (MIF) – The following abstract was accepted as a poster at the American Heart Association Scientific Sessions 2012 Meeting in Los Angeles:

- Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. American Heart Association Scientific Sessions 2012, November 3-7, 2012, Los Angeles, CA.

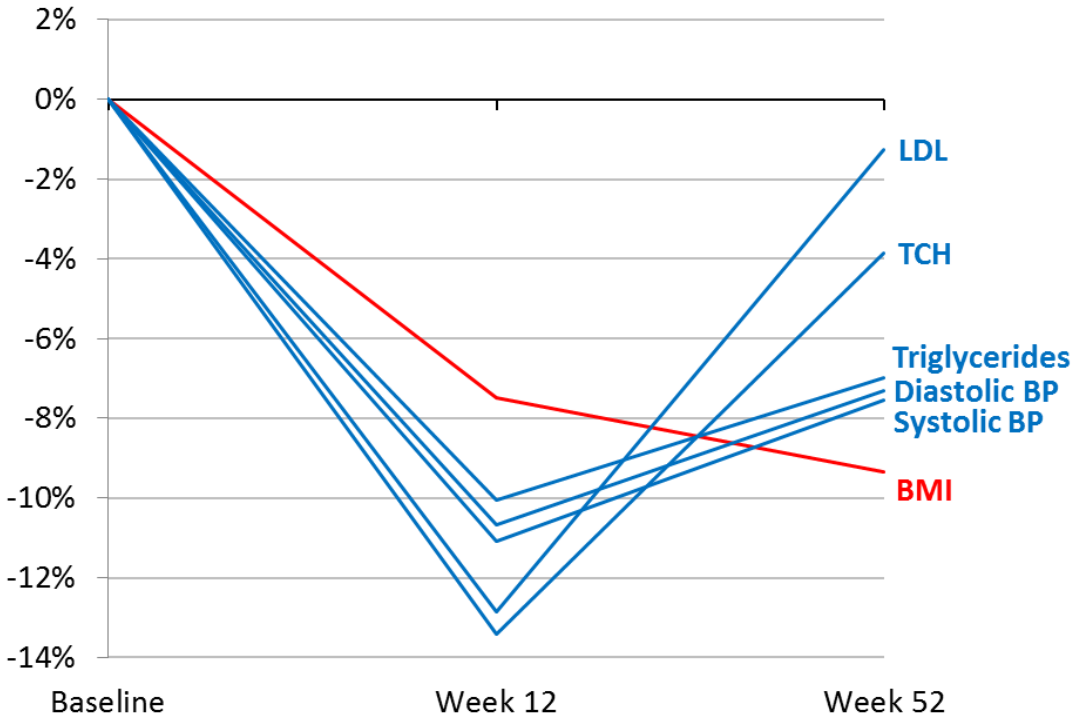
Gene Expression – Pathway analysis was performed on Ornish vs Control datasets, comparing each time point vs control, using three different pathway databases, KEGG, BioCarta, and Broad Molecular Signature Data Base. For the BioCarta database, this analysis identified 3 pathways differentially expressed at 3 months and 5 pathways differentially expressed at 1 year in Ornish participants. The KEGG pathways are:

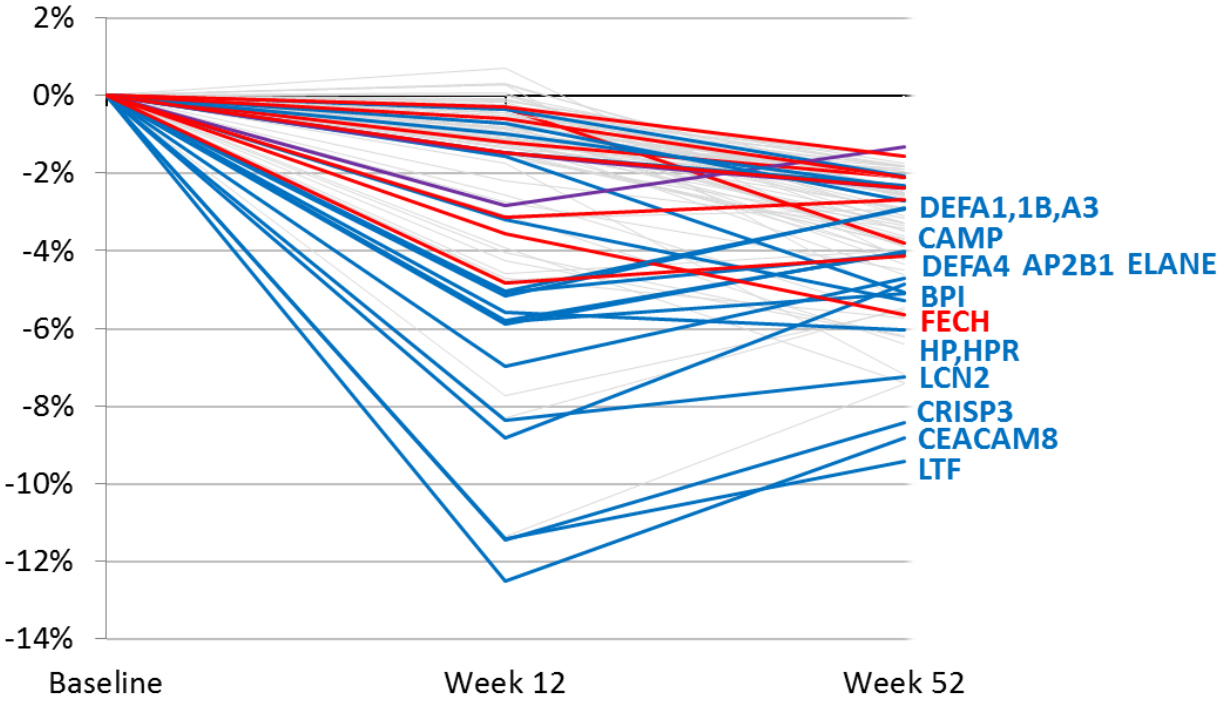
Baseline – Week 12		Genes	
hsa00640	Propanoate metabolism	26	Carboxylic acid metabolism; related to carbohydrate metabolism
hsa04912	GnRH signaling pathway	64	Synthesis/release of gonadotropins; gene expression, cell proliferation
hsa05120	Epithelial cell signaling in Hpy	53	Human diseases; Infectious diseases
Baseline – Week 52			
hsa00150	Androgen and estrogen metabolism	19	Inactivation/catabolism of androgen & estrogen in target tissues
hsa04810	Regulation of actin cytoskeleton	136	Cellular processes; Cell motility
hsa00563	GPI-anchor biosynthesis	18	Metabolism; Glycan biosynthesis/metabolism

Manuscript nearing completion. Additional analysis showed that changes in gene expression mirrored changes in many CVD risk factors (Fig. 1) – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52 (Fig. 2). Most cholesterol and lipid homeostasis genes showed a continual decrease in expression throughout the program similar to body weight (Fig. 2). Medication use clearly did not affect gene expression, thus expression changes may be attributed to the lifestyle change program (Table 1).

Abstract: accepted as a *poster presentation:*

- Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. The importance of weight loss for effecting molecular change during intensive cardiovascular risk reduction. Obesity 2012: 30th Annual Scientific Meeting, September 20-24, 2012, San Antonio, TX.





Figures 1 and 2. Change in traditional CVD risk factors (Fig. 1) and changes in gene expression (Fig. 2) during intensive lifestyle change.

Table 1. Effects of medications on gene expression from Baseline to Week 52

Probe ID	Symbol	Fold Change All Participants (n=63)	Fold Change Lipid Lowering Medications* (n=51)	Fold Change All Medications† (n=34)
202018_s_at	LTF	–1.67	–1.67	–1.70
221748_s_at	TNS1	–1.55	–1.51	–1.43
212531_at	LCN2	–1.47	–1.44	–1.48
206676_at	CEACAM8	–1.44	–1.48	–1.68
214407_x_at	GYPB	–1.41	–1.34	–1.26
206698_at	XK	–1.41	–1.43	–1.36
206665_s_at	BCL2L1	–1.39	–1.35	–1.31
203502_at	BPGM	–1.37	–1.40	–1.41
203115_at	FECH	–1.35	–1.31	–1.28
207802_at	CRISP3	–1.32	–1.32	–1.43
208470_s_at	HP/HPR	–1.30	–1.31	–1.24
212768_s_at	OLFM4	–1.29	–1.20	–1.23
213446_s_at	IQGAP1	–1.28	–1.25	–1.22
208632_at	RNF10	–1.28	–1.25	–1.18
221627_at	TRIM10	–1.28	–1.23	–1.21
218418_s_at	KANK2	–1.28	–1.22	–1.21
217878_s_at	CDC27	–1.27	–1.26	–1.22
210244_at	CAMP	–1.27	–1.26	–1.27

200615_s_				–1.22
at	AP2B1	–1.26	–1.24	
205557_at	BPI	–1.25	–1.22	–1.29
211993_at	WNK1	–1.25	–1.23	–1.17

Plasma Metabolites – Collaboration with Dr. Dean Jones and Dr. Quinlyn Soltow at Emory University to profile plasma metabolites associated with CVD development continuing. We have analyzed 17 Ornish patients and 17 matched controls (all at three time points) by liquid chromatography-Fourier transform mass spectrometry (LC-FTMS). All assays run in duplicate, manuscript in preparation.

Structural and Functional Measures of Cardiovascular Health

Specific endpoints measured include ejection fraction and wall motion, coronary artery calcification scores, left and right ventricular volumes, myocardial mass, stenosis sizing and vessel diameter, plaque density and differentiation of calcified versus non-calcified plaque, and tissue perfusion and viability. Work continues on the quantification and interpretation of the huge volumes of imaging data we have acquired.

Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal, Sub-Study for Previous Subjects in the Dr. Dean Ornish Program for Reversing Heart Disease.

The primary objective of this study is to examine associations between DNA variation (in the form of 500,000+ single nucleotide polymorphisms) and participant response to the program. We are examining the influence of innate genetic variation on overall response, quantified as the risk of future cardiac events (Framingham risk), as well as response of specific cardiovascular disease risk factors. The main hypothesis is that innate variation in genes associated with lipid metabolism, protein biosynthesis, protein modification, transcription regulation and/or cell surface receptors (or other genes) will correlate positively with response to intensive lifestyle changes involving diet, exercise, meditation, yoga and group support, which may lead to improved CHD risk factor profiles and genetic markers of coronary artery disease reversal or stabilization. Participants in this study are being recruited from previous cohorts of the Dr. Dean Ornish Program for Reversing Heart Disease at Windber Medical Center (prior to implementation of the primary Molecular Profiling Protocol described above).

Status:

Profiled 39 SNPs defined in recent genome-wide association studies to have an impact on CVD development or associated risk factors; influence of 23 SNPs on triglyceride response has been evaluated; manuscript nearly complete, data analysis is ongoing.

Four additional SNPs were genotyped, 3 in close proximity to rs17145738 and one near rs3846662. rs17145738 and rs3846662 showed significant differences between genotypes in triglyceride response in previous analyses. Of the 4 new SNPs, 2 SNPs (rs12916 and rs714052) also showed a significant difference in triglyceride response to the Ornish program between genotypes. The following abstract was accepted for poster presentation at the ASHG Meeting in San Francisco:

- Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence

response during intensive cardiovascular risk reduction. American Society of Human Genetics Annual Meeting, November 6-10, 2012, San Francisco, CA.

Dietary Factors in the Ornish and CRC Programs – Brianne Seitz our summer intern compared dietary outcomes of CRC participants to the Ornish program and controls. Ornish participants showed improvement in most dietary factors, but there was little change in controls. Ornish participants significantly lowered their daily fat intake by more than 60% (P<0.001 versus controls), while increasing carbohydrate intake by 30% (P<0.001 versus controls). Due to the stringency of the program, initial changes among Ornish participants were larger than those in CRC participants, but there was evidence that CRC participants showed less regression over time.

Task #11: Cardiovascular Risk Assessment and Prevention Program.

Status:
Study is currently ongoing.

Background:
This program, now called the Cardiovascular Risk Clinic, is being established as a platform to address the unique needs of retired military beneficiaries at risk for CV disease. The program mirrors the Cardiac Prevention Program (CPP) designed and established by the ICHP at WRAMC. It includes conventional and novel CV risk profiling and tailored, personalized behavioral recommendations for primary or secondary prevention by an integrative team of providers comprised of a nurse case manager, psychologist, nurse practitioners, dietitians, stress management instructors, and exercise physiologists. Validated tools to screen for and measure CV risk, stress, sleep health, compliance with dietary recommendations and exercise are standard of care. The program is an adjunct to the best medical practices provided by their primary care provider.

Phase I of the program involves each participant undergoing a comprehensive health risk assessment that is completed by a physician, followed by a four- hour “Pearls for your Heart” workshop and participants then schedule individual appointments with each modality specialist to receive education and counseling in nutrition, exercise, stress management and mind/body health. These are monthly appointments to be completed over a 4-6 month period.

Phase II of the program begins after the completion of the healthy lifestyle intervention (Phase I). During this phase each participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the Program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase 2 for five years, during which time they will come to the center for re-assessments every six months.

Subject Enrollment and Demographics:
Total subject enrollment in the CRC is 216 participants; 181 remain active (127 intervention; 89 controls); 35 drop-outs; 28 control participants have transferred to the intervention arm after one year as a control. Demographic characteristics of participants are: average age 58.9 years, 58% female, 22% veterans or the spouse of a veteran, and 20% with diagnosed coronary heart disease.

In the last quarter (July 2012- 15 Sept 2012) there were a total of 394 participant visits including periodic follow up phone calls to participants enrolled in the intervention arm of the study and 114 visits by participants enrolled in the control arm of the study.

Outcome Data
The intervention cohorts have shown change in the desired direction for virtually all of the measured coronary artery disease (CAD) risk factors over the initial 4-6 month period (Table 2A). Measures of obesity including weight and BMI declined ~3.5%. Levels of total cholesterol were reduced by ~2%, and triglycerides dropped by 13%. Dietary analysis shows marked improvement in daily total and saturated fat intake, two main dietary components that contribute to plaque formation. Systolic and diastolic blood pressure decreased by nearly 7%. Measures of carotid intimal-medial thickness (CIMT) also were significantly lower after the intervention phase. In addition, psychometric measures also significantly decreased, specifically, depression by 30% and hostility by 15%. Similarly, sleep quality improved by 25%. This data demonstrates that lifestyle change programs may be important for primary prevention in individuals with diagnosed CAD and those at increased risk of disease.

Results from the first long-term follow up time point (6 months after completion of the intervention) are shown in Table 2B. Over the course of approximately 8-10 months, weight, BMI, total cholesterol, triglycerides, blood pressure, and CIMT measurements all maintained statistical significance proving that the positive improvements in these traditional risk factors for CAD can be maintained over a longer period of time. In addition, at this time point, depression and hostility remained significantly improved as well sleep quality.

In Table 2C, results 1 year after completion of the intervention are shown. Weight, BMI, total cholesterol, LDL cholesterol, CIMT measurements as well as psychosocial and sleep factors continue to maintain statistical significance. Most variables continue to trend in the desirable direction.

Tables 2D and 2E show the furthest time points reached thus far, 18 months and 2 years respectively after completion of the intervention. Although a relatively small sample size risk factors continue to show positive improvements.

Table 2A. Comparison of "Baseline" to "Intervention Complete" (4-6 months) data for participants in the intervention arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Intervention Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	91	194.98 (45.6)	190.48 (42.8)	-4.5	<0.00001

Body Mass Index	88	30.92 (6.1)	30.16 (6.0)	-0.8	<0.00001
Total Cholesterol (mg/dl)	85	187.59 (39.4)	184.28 (37.5)	-3.3	0.2845
High Density Lipids (mg/dl)	85	48.42 (12.6)	48.74 (11.1)	0.3	0.7003
Low Density Lipids (mg/dl)	85	112.49 (32.0)	111.98 (31.4)	-0.5	0.8370
Triglycerides (mg/dl)	85	135.40 (72.3)	117.69 (51.0)	-17.7	<0.001
Systolic Blood Pressure	85	131.48 (17.9)	125.06 (17.0)	-6.4	<0.0001
Diastolic Blood Pressure	85	80.64 (11.1)	75.48 (9.2)	-5.2	<0.00001
Depression Scale [CES-D]	85	10.32 (9.3)	7.11 (6.9)	-3.2	<0.00001
Hostility Scale [Cook-Medley]	85	7.26 (4.7)	6.20 (4.2)	-1.1	<0.001
Perceived Stress Scale [PSS]	85	13.09 (5.9)	10.73 (5.4)	-2.4	<0.0001
Daily Total Fat (grams)	79	64.05 (32.6)	52.43 (22.6)	-11.6	<0.01
Daily Saturated Fat (grams)	79	19.85 (10.7)	16.38 (8.8)	-3.5	<0.01
Avg. CCA/Mean IMT	85	0.748 (0.1608)	0.700 (0.1407)	-0.048	<0.00001
Avg. CCA / Max IMT	85	0.862 (0.1856)	0.805 (0.1506)	-0.1	<0.00001
Fasting Glucose (mg/dl)	85	104 (33.6)	101 (24.4)	-3.0	0.2996
HgbA1c	85	6.0 (1.07)	6.0 (1.10)	-0.1	0.2433
Cortisol	84	11.9 (3.93)	13.7 (3.98)	1.8	<0.001
TSH	85	1.94 (1.063)	2.14 (1.305)	0.2	0.1285
Epworth Sleepiness Scale (0 to 24)	84	8 (4.5)	7 (4.2)	-0.9	<0.01
Pittsburgh Sleep Quality Index (0-21)	83	8 (4.3)	6 (3.9)	-1.3	<0.0001

Table 2B. Change in outcome variables 6 months after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	60	190.84 (45.1)	184.07 (41.2)	-6.8	<0.00001
Body Mass Index	60	30.47 (6.0)	29.59 (5.7)	-0.9	<0.001
Total Cholesterol (mg/dl)	61	189.41 (42.4)	178.25 (38.1)	-11.2	<0.05
High Density Lipids (mg/dl)	61	49.28 (13.7)	48.44 (12.8)	-0.8	0.3427
Low Density Lipids (mg/dl)	61	112.38 (32.8)	105.56 (31.5)	-6.8	0.0896
Triglycerides (mg/dl)	61	141.64 (72.7)	122.25 (65.6)	-19.4	<0.01
Systolic Blood Pressure	60	131.03 (18.0)	126.40 (17.3)	-4.6	<0.05
Diastolic Blood Pressure	60	80.50 (11.0)	74.97 (9.1)	-5.5	<0.001
Depression Scale [CES-D]	59	9.37 (9.3)	7.12 (9.1)	-2.3	<0.05

Hostility Scale [Cook-Medley]	59	7.00 (4.6)	6.56 (4.3)	-0.4	0.2956
Perceived Stress Scale [PSS]	59	12.47 (6.1)	10.44 (6.6)	-2.0	<0.01
Daily Total Fat (grams)	55	64.33 (33.0)	51.42 (20.8)	-12.9	<0.05
Daily Saturated Fat (grams)	55	19.56 (10.6)	15.79 (8.1)	-3.8	<0.05
Avg. CCA/Mean IMT	60	0.772 (0.1446)	0.699 (0.1407)	-0.073	<0.00001
Avg. CCA / Max IMT	60	0.891 (0.1680)	0.798 (0.1508)	-0.1	<0.00001
Fasting Glucose (mg/dl)	62	102 (20.0)	102 (25.5)	0.5	0.8710
HgbA1c	61	6.1 (0.94)	6.0 (1.19)	-0.1	0.1140
Cortisol	59	11.8 (3.96)	13.6 (4.02)	1.8	<0.01
TSH	61	1.99 (1.180)	2.31 (1.527)	0.3	<0.05
Epworth Sleepiness Scale (0 to 24)	57	8 (4.4)	7 (4.3)	-1.1	<0.01
Pittsburgh Sleep Quality Index (0-21)	57	7 (4.0)	6 (3.6)	-1.4	<0.001

Table 2C. Change in outcome variables 1 year after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	27	191.01 (41.7)	183.30 (38.3)	-7.7	<0.0001
Body Mass Index	26	30.47 (5.7)	29.24 (5.1)	-1.2	<0.001
Total Cholesterol (mg/dl)	26	187.96 (41.0)	171.38 (37.9)	-16.6	<0.01
High Density Lipids (mg/dl)	26	52.50 (15.6)	50.46 (13.2)	-2.0	0.2047
Low Density Lipids (mg/dl)	26	109.81 (30.8)	98.65 (27.6)	-11.2	<0.01
Triglycerides (mg/dl)	26	131.12 (70.1)	111.81 (54.6)	-19.3	0.0563
Systolic Blood Pressure	26	132.15 (14.0)	125.85 (14.1)	-6.3	0.0591
Diastolic Blood Pressure	26	79.54 (10.2)	75.54 (7.8)	-4.0	0.0686
Depression Scale [CES-D]	26	10.00 (9.7)	6.92 (7.4)	-3.1	<0.05
Hostility Scale [Cook-Medley]	26	7.96 (4.5)	6.69 (4.2)	-1.3	<0.05
Perceived Stress Scale [PSS]	26	12.77 (7.1)	10.23 (6.3)	-2.5	<0.01
Daily Total Fat (grams)	26	69.24 (32.2)	45.08 (13.8)	-24.2	<0.001
Daily Saturated Fat (grams)	26	20.91 (10.9)	13.31 (4.8)	-7.6	<0.01
Avg. CCA/Mean IMT	26	0.860 (0.1249)	0.719 (0.1172)	-0.141	<0.00001
Avg. CCA / Max IMT	26	0.996 (0.1510)	0.828 (0.1365)	-0.2	<0.00001
Fasting Glucose (mg/dl)	26	101 (13.7)	104 (22.3)	2.7	0.4426
HgbA1c	26	6.2 (1.05)	6.0 (1.21)	-0.2	<0.01
Cortisol	26	12.1 (4.57)	13.9 (3.07)	1.8	<0.05
TSH	26	2.34 (1.387)	2.39 (1.371)	0.0	0.8242
Epworth Sleepiness Scale (0 to	25	8 (4.3)	7 (3.6)	-1.1	0.1034

24)					
Pittsburgh Sleep Quality Index (0-21)	25	9 (5.0)	6 (3.2)	-2.2	<0.01

Table 2D. Change in outcome variables 18 months after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	14	188.96 (42.7)	182.93 (38.0)	-6.0	<0.05
Body Mass Index	14	29.56 (4.2)	28.44 (3.8)	-1.1	<0.01
Total Cholesterol (mg/dl)	14	179.14 (46.2)	175.29 (43.6)	-3.9	0.6836
High Density Lipids (mg/dl)	14	52.36 (10.8)	49.21 (7.2)	-3.1	0.2554
Low Density Lipids (mg/dl)	14	105.50 (35.6)	105.71 (32.6)	0.2	0.9780
Triglycerides (mg/dl)	14	112.71 (52.7)	101.00 (41.5)	-11.7	0.2346
Systolic Blood Pressure	14	128.29 (14.5)	129.86 (20.1)	1.6	0.7470
Diastolic Blood Pressure	14	77.86 (11.9)	74.00 (8.5)	-3.9	0.1917
Depression Scale [CES-D]	14	11.00 (11.2)	10.36 (10.7)	-0.6	0.7789
Hostility Scale [Cook-Medley]	14	7.14 (4.6)	6.57 (4.2)	-0.6	0.5616
Perceived Stress Scale [PSS]	14	12.21 (8.1)	12.36 (8.7)	0.1	0.9482
Daily Total Fat (grams)	14	58.69 (30.7)	63.48 (52.2)	4.8	0.7661
Daily Saturated Fat (grams)	14	18.75 (12.2)	24.69 (27.5)	5.9	0.4872
Avg. CCA/Mean IMT	13	0.853 (0.1176)	0.698 (0.1431)	-0.156	<0.00001
Avg. CCA / Max IMT	13	0.982 (0.1184)	0.809 (0.1548)	-0.2	<0.00001
Fasting Glucose (mg/dl)	14	101 (12.3)	103 (33.7)	2.4	0.7658
HgbA1c	14	6.3 (1.16)	6.1 (1.21)	-0.2	<0.01
Cortisol	14	13.0 (4.66)	13.2 (4.60)	0.2	0.9239
TSH	14	2.18 (1.366)	2.24 (1.724)	0.1	0.8444
Epworth Sleepiness Scale (0 to 24)	14	7 (4.6)	6 (4.4)	-1.0	0.2544
Pittsburgh Sleep Quality Index (0-21)	14	8 (4.9)	6 (3.0)	-2.1	<0.05

Table 2E. Change in outcome variables 2 years after completion of the intervention for participants in the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average 10 month value (SD)	Average Change	P-Value
Weight (lbs.)	14	188.96 (42.7)	183.63 (39.7)	-5.3	<0.05
Body Mass Index	14	29.56 (4.2)	28.71 (4.0)	-0.8	<0.05
Total Cholesterol (mg/dl)	14	179.14	174.64 (48.2)	-4.5	0.7302

		(46.2)			
High Density Lipids (mg/dl)	14	52.36 (10.8)	50.21 (8.0)	-2.1	0.3804
Low Density Lipids (mg/dl)	14	105.50 (35.6)	106.57 (37.8)	1.1	0.9158
Triglycerides (mg/dl)	14	112.71 (52.7)	89.86 (51.3)	-22.9	0.0637
Systolic Blood Pressure	14	128.29 (14.5)	126.00 (16.2)	-2.3	0.6571
Diastolic Blood Pressure	14	77.86 (11.9)	73.29 (8.6)	-4.6	0.1246
Depression Scale [CES-D]	14	11.00 (11.2)	7.93 (9.8)	-3.1	0.0673
Hostility Scale [Cook-Medley]	14	7.14 (4.6)	6.07 (4.3)	-1.1	0.1969
Perceived Stress Scale [PSS]	14	12.21 (8.1)	9.43 (7.2)	-2.8	0.0565
Daily Total Fat (grams)	14	58.69 (30.7)	63.44 (18.8)	4.8	0.6025
Daily Saturated Fat (grams)	14	18.75 (12.2)	19.69 (8.1)	0.9	0.8048
Avg. CCA/Mean IMT	14	0.846 (0.1166)	0.650 (0.1460)	-0.196	<0.00001
Avg. CCA / Max IMT	14	0.974 (0.1180)	0.750 (0.1617)	-0.2	<0.00001
Fasting Glucose (mg/dl)	14	101 (12.3)	105 (31.5)	4.1	0.5713
HgbA1c	14	6.3 (1.16)	5.7 (1.07)	-0.6	<0.001
Cortisol	14	13.0 (4.66)	13.8 (2.98)	0.7	0.5582
TSH	14	2.18 (1.366)	2.83 (2.280)	0.6	0.0592
Epworth Sleepiness Scale (0 to 24)	14	7 (4.6)	5 (3.5)	-1.9	<0.05
Pittsburgh Sleep Quality Index (0-21)	14	8 (4.9)	6 (3.7)	-2.1	<0.05

In subjects randomized to the control arm of the study, who do not participate in the lifestyle change intervention showed no significant changes in risk factors, except for CIMT and cortisol at the first 6 month time point (Table 3A). Subsequent follow up time points (Table 3B: 6 month time point; Table 3C: 1 year; Table 3D: 18 months; and Table 3E: 2 years) continue to show that most risk factors did not improve and those risk factors that did not maintain improvement at the next time point, perhaps this improvement could be attributed to small sample size and large variability among the participant's results. This lack of consistent improvement within the control arm further proves the benefits of a team-base, patient-centered lifestyle change model in improving risk for developing heart disease.

Table 3A. Change in outcome variables from baseline to "waiting period complete" period for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	67	189.08 (43.0)	188.63 (42.5)	-0.4	0.6694
Body Mass Index	67	30.97 (6.4)	30.84 (6.2)	-0.1	0.6791

Total Cholesterol (mg/dl)	68	190.87 (37.0)	186.60 (35.9)	-4.3	0.2413
High Density Lipids (mg/dl)	68	49.85 (14.5)	49.07 (12.3)	-0.8	0.3747
Low Density Lipids (mg/dl)	68	115.50 (31.2)	110.56 (31.0)	-4.9	0.1406
Triglycerides (mg/dl)	68	130.40 (65.5)	133.00 (68.5)	2.6	0.6591
Systolic Blood Pressure	68	129.85 (17.8)	130.59 (21.0)	0.7	0.7205
Diastolic Blood Pressure	68	79.06 (10.2)	77.85 (10.2)	-1.2	0.2927
Depression Scale [CES-D]	67	11.61 (10.2)	10.61 (8.9)	-1.0	0.2772
Hostility Scale [Cook-Medley]	67	7.60 (4.9)	7.30 (5.1)	-0.3	0.3442
Perceived Stress Scale [PSS]	67	13.33 (7.1)	13.10 (7.7)	-0.2	0.7121
Daily Total Fat (grams)	57	72.24 (32.3)	68.37 (32.7)	-3.9	0.4075
Daily Saturated Fat (grams)	57	22.25 (10.2)	21.14 (11.2)	-1.1	0.4542
Avg. CCA / Mean IMT	67	0.801 (0.2040)	0.746 (0.1778)	-0.055	<0.001
Avg. CCA / Max IMT	67	0.924 (0.2406)	0.850 (0.1990)	-0.1	<0.0001
Fasting Glucose (mg/dl)	68	108 (37.4)	109 (41.2)	0.3	0.9243
HgbA1c	67	6.0 (1.32)	6.0 (1.10)	0.0	0.8779
Cortisol	68	12.0 (4.31)	13.6 (4.56)	1.6	<0.001
TSH	66	1.99 (1.253)	2.07 (1.088)	0.1	0.5620
Epworth Sleepiness Scale (0 to 24)	66	8 (4.3)	8 (3.9)	-0.4	0.4011
Pittsburgh Sleep Quality Index (0-21)	66	7 (3.6)	7 (3.5)	-0.2	0.5784

Table 3B. Change in outcome variables at the 6 month time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	50	194.80 (43.7)	194.68 (45.7)	-0.1	0.9370
Body Mass Index	49	31.63 (6.7)	31.41 (6.8)	-0.2	0.4642
Total Cholesterol (mg/dl)	50	194.94 (33.8)	183.84 (34.0)	-11.1	<0.01
High Density Lipids (mg/dl)	50	51.06 (15.2)	49.30 (17.6)	-1.8	0.3313
Low Density Lipids (mg/dl)	50	117.80 (27.7)	110.54 (27.8)	-7.3	<0.05
Triglycerides (mg/dl)	50	133.98 (65.0)	128.32 (57.8)	-5.7	0.4946
Systolic Blood Pressure	50	130.48 (16.2)	131.00 (24.8)	0.5	0.8496
Diastolic Blood Pressure	50	79.16 (10.4)	77.76 (11.8)	-1.4	0.4013
Depression Scale [CES-D]	48	10.98 (9.8)	11.02 (9.9)	0.0	0.9692

Hostility Scale [Cook-Medley]	48	7.40 (5.2)	7.06 (4.6)	-0.3	0.3528
Perceived Stress Scale [PSS]	48	12.48 (7.2)	12.21 (7.0)	-0.3	0.7484
Daily Total Fat (grams)	47	71.80 (34.2)	65.51 (28.4)	-6.3	0.2617
Daily Saturated Fat (grams)	47	22.03 (10.8)	22.19 (10.5)	0.2	0.9265
Avg. CCA / Mean IMT	48	0.847 (0.1892)	0.740 (0.1719)	-0.107	<0.00001
Avg. CCA / Max IMT	48	0.976 (0.2225)	0.855 (0.1923)	-0.1	<0.0001
Fasting Glucose (mg/dl)	50	115 (41.5)	114 (41.6)	-0.6	0.8686
HgbA1c	50	6.2 (1.43)	6.2 (1.19)	-0.1	0.4052
Cortisol	50	12.0 (4.08)	13.1 (3.98)	1.1	0.0686
TSH	50	1.96 (1.267)	2.12 (0.935)	0.2	0.3041
Epworth Sleepiness Scale (0 to 24)	48	8 (4.2)	8 (3.9)	-0.1	0.8213
Pittsburgh Sleep Quality Index (0-21)	48	6 (3.5)	6 (3.4)	0.1	0.7277

Table 3C. Change in outcome variables at year 1 time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	22	199.55 (41.6)	194.65 (42.3)	-4.9	<0.05
Body Mass Index	22	31.75 (5.9)	30.68 (6.1)	-1.1	<0.05
Total Cholesterol (mg/dl)	21	196.86 (39.3)	182.33 (37.8)	-14.5	<0.05
High Density Lipids (mg/dl)	21	54.38 (19.0)	49.86 (17.0)	-4.5	<0.01
Low Density Lipids (mg/dl)	21	116.43 (32.6)	107.43 (30.9)	-9.0	0.2162
Triglycerides (mg/dl)	21	138.71 (81.7)	124.76 (77.1)	-14.0	0.1953
Systolic Blood Pressure	21	131.24 (14.8)	129.62 (19.6)	-1.6	0.6637
Diastolic Blood Pressure	21	80.76 (9.8)	77.81 (12.5)	-3.0	0.1814
Depression Scale [CES-D]	21	9.48 (9.4)	9.43 (10.4)	0.0	0.9682
Hostility Scale [Cook-Medley]	21	7.76 (5.5)	7.95 (4.7)	0.2	0.7558
Perceived Stress Scale [PSS]	21	11.52 (8.0)	12.24 (6.6)	0.7	0.5529
Daily Total Fat (grams)	16	80.18 (33.7)	67.22 (35.2)	-13.0	0.1789
Daily Saturated Fat (grams)	16	24.46 (10.0)	21.61 (10.9)	-2.8	0.3257
Avg. CCA / Mean IMT	21	0.914 (0.1655)	0.774 (0.1364)	-0.141	<0.00001
Avg. CCA / Max IMT	21	1.057 (0.1763)	0.882 (0.1520)	-0.2	<0.00001
Fasting Glucose (mg/dl)	21	121 (46.6)	111 (47.1)	-9.9	0.1832
HgbA1c	21	6.4 (1.46)	6.1 (1.00)	-0.4	0.0886
Cortisol	21	13.7 (4.34)	13.3 (4.50)	-0.4	0.7584
TSH	21	1.84 (1.132)	2.03 (0.822)	0.2	0.2164

Epworth Sleepiness Scale (0 to 24)	21	9 (4.7)	7 (4.1)	-1.1	0.1530
Pittsburgh Sleep Quality Index (0-21)	21	7 (4.1)	6 (3.6)	-0.2	0.6863

Table 3D. Change in outcome variables 18 month time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value
Weight (lbs.)	13	204.22 (40.8)	198.86 (47.3)	-5.4	0.3780
Body Mass Index	13	33.15 (6.0)	32.19 (7.0)	-1.0	0.3696
Total Cholesterol (mg/dl)	13	211.00 (35.7)	201.31 (40.9)	-9.7	0.4169
High Density Lipids (mg/dl)	13	56.77 (21.2)	54.92 (20.4)	-1.8	0.4082
Low Density Lipids (mg/dl)	13	129.00 (31.0)	120.69 (35.6)	-8.3	0.5152
Triglycerides (mg/dl)	13	136.08 (67.7)	127.92 (98.0)	-8.2	0.6172
Systolic Blood Pressure	9	134.89 (17.0)	127.33 (24.5)	-7.6	0.3164
Diastolic Blood Pressure	9	81.33 (10.5)	79.78 (9.6)	-1.6	0.6560
Depression Scale [CES-D]	12	8.00 (5.0)	9.00 (7.6)	1.0	0.5124
Hostility Scale [Cook-Medley]	12	6.92 (5.1)	7.00 (5.2)	0.1	0.9030
Perceived Stress Scale [PSS]	12	10.58 (5.6)	11.75 (6.3)	1.2	0.5632
Daily Total Fat (grams)	10	72.35 (27.3)	80.50 (31.1)	8.1	0.4831
Daily Saturated Fat (grams)	10	21.82 (6.9)	25.60 (8.5)	3.8	0.2724
Avg. CCA / Mean IMT	13	0.935 (0.1549)	0.751 (0.1475)	-0.183	<0.0001
Avg. CCA / Max IMT	13	1.069 (0.1786)	0.862 (0.1681)	-0.2	<0.0001
Fasting Glucose (mg/dl)	13	113 (38.6)	104 (21.8)	-9.2	0.2277
HgbA1c	13	6.4 (1.31)	5.9 (0.69)	-0.5	0.0673
Cortisol	13	13.3 (4.52)	11.4 (4.93)	-1.9	0.2866
TSH	13	1.65 (0.836)	1.92 (0.564)	0.3	0.1088
Epworth Sleepiness Scale (0 to 24)	12	9 (4.8)	7 (4.0)	-1.4	0.1857
Pittsburgh Sleep Quality Index (0-21)	12	7 (4.7)	7 (4.3)	-0.5	0.6595

Table 3E. Change in outcome variables at Year 2 time point for participants in the control arm of the Cardiovascular Risk Clinic

Category / Metrics	N	Average Baseline Value (SD)	Average Waiting Period Complete Value (SD)	Average Change	P-Value

Weight (lbs.)	8	192.63 (43.1)	186.48 (38.6)	-6.2	<0.05
Body Mass Index	9	32.43 (5.9)	30.80 (5.3)	-1.6	0.0917
Total Cholesterol (mg/dl)	8	209.50 (36.3)	182.75 (48.2)	-26.8	0.1127
High Density Lipids (mg/dl)	8	62.75 (25.4)	56.88 (22.9)	-5.9	0.1441
Low Density Lipids (mg/dl)	8	122.38 (25.2)	99.50 (35.3)	-22.9	0.2084
Triglycerides (mg/dl)	8	137.50 (82.4)	131.75 (64.5)	-5.8	0.7400
Systolic Blood Pressure	8	135.50 (17.3)	135.50 (25.8)	0.0	1.0000
Diastolic Blood Pressure	8	82.75 (10.3)	73.13 (10.3)	-9.6	<0.01
Depression Scale [CES-D]	6	5.00 (3.2)	4.00 (3.6)	-1.0	0.4466
Hostility Scale [Cook-Medley]	6	7.50 (4.1)	9.00 (4.4)	1.5	0.2264
Perceived Stress Scale [PSS]	6	9.50 (7.0)	8.50 (6.4)	-1.0	0.6884
Daily Total Fat (grams)	3	58.89 (19.6)	79.45 (16.9)	20.6	0.0850
Daily Saturated Fat (grams)	3	17.28 (6.3)	29.19 (11.2)	11.9	0.1616
Avg. CCA / Mean IMT	8	0.915 (0.1560)	0.694 (0.1393)	-0.221	<0.001
Avg. CCA / Max IMT	8	1.037 (0.1771)	0.775 (0.1518)	-0.3	<0.001
Fasting Glucose (mg/dl)	8	121 (45.9)	107 (20.2)	-14.3	0.2917
HgbA1c	8	6.4 (1.27)	5.8 (0.65)	-0.7	0.0740
Cortisol	8	12.8 (5.25)	12.4 (3.65)	-0.5	0.8034
TSH	8	1.61 (0.804)	2.54 (1.447)	0.9	0.2154
Epworth Sleepiness Scale (0 to 24)	6	9 (4.0)	8 (4.2)	-1.2	0.3522
Pittsburgh Sleep Quality Index (0-21)	6	5 (2.1)	6 (3.4)	0.3	0.7497

Adverse Events

All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There were no adverse events in either arm of the study during the last quarter. To date there have been a total of 16 adverse events, 8 in the intervention and 8 in the control arm of the study, all deemed serious events, not related and not expected. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Therefore, all 16 events were considered serious due to inpatient hospitalizations. There were 7 non-cardiac and 1 cardiac adverse events in the intervention arm of the study. No deaths occurred and none of these adverse events were deemed to be study related. There were 5 non-cardiac and 3 cardiac adverse events in the control arm of the study. No deaths occurred and none of these adverse events were deemed to be study related.

NMR Lipid Panel and Biomarkers
This year for the CRC program, approximately 5,570 aliquots have been made summarized by the following:

PAXGene Tubes	296
RBCs	586

Plasma samples		Serum samples	
NMR lipids	293	Adiponectin	298
Leptin	293	Serum amyloid A	298
CRP	293	Vitamin D	298
Resistin	293	Lp(a)	298
Insulin	293	Extra Serum	1165
Extra plasma	866		

Task #11a: Initiate Stress Therapy Empowering Prevention (STEP) component to the Cardiovascular Risk Assessment program outlined in Task #11.

Status:
Study is currently being closed for enrollment but will remain open for data analysis.

Background:
This is a collaborative study involving researchers from Windber Research Institute and Walter Reed Army Medical Center and is modeled after the Caretakers Optimizing Readiness through Preventive Strategies (CORPS), designed by the Integrative Cardiac Health Program (ICHP) at Walter Reed Army Medical Center (WRAMC), except that it targets participants with chronic disease. The purpose of this task is to determine the degree of stress, sleep disturbance, and cardiovascular disease risk in patients who have been diagnosed with breast cancer or are at high risk of developing breast disease.

In the first part of the intervention, patients will be randomized to a 12 week Healthy Lifestyle intervention group or a non-intervention group. During this phase, each intervention participant undergo a comprehensive health risk assessment that is completed by a physician, followed by mandatory attendance to on-site group sessions in which they will participate in 1 hour of stress management, 30 minutes of nutrition education every week, and 30 minutes of exercise alternated with 30 minutes of mind/body health every other week. In addition, the nurse will provide educational lectures on various health topics during 4 sessions. After completing Phase I, patients will participate in a five year healthy lifestyle intervention or control group.

During phase II each intervention participant will again meet with the physician. During this appointment the physician will prepare the participants for the next phase and give them strategies for maintaining success on their own. The second phase of the program provides additional reinforcement through monthly phone calls with an integrative health coach. Participants will remain in Phase II for five years, during which time they will come to the center for re-assessments every six months.

We hypothesize that the 12 week healthy lifestyle interventions will significantly reduce stress, sleep disturbances, and cardiovascular risk in patients at risk for, or already diagnosed with, breast cancer.

Subject Enrollment and Demographics:

Total subject enrollment was 18 (intervention only); 10 active; 8 dropouts. Demographic characteristics of participants were: average age 65.6 years, 28% veterans or the spouse of a veteran, 6% have diagnosed coronary heart disease, and 61% have diagnosed breast cancer. Due to the lack of public interest we were unable to recruit a sufficient number of participants to keep this protocol open. The protocol was closed for enrollment on September 1, 2012 but will remain open for data analysis.

In the last quarter (July 2012- 15 Sept 2012) there were a total of 8 participant visits including periodic follow up phone calls made to participants.

Outcomes Data:

Overall participants showed change in the desired direction for most of the measured coronary artery disease (CAD) risk factors over the 2 years of the program (see Tables 4A-4D below). No participants were enrolled into the control arm of the study and lack of statistically significant levels of improvement in some measures may be attributable to small sample size and wide variability in some measures.

Table 4A. Comparison of baseline to Week 12 data for participants in the STEP Program

Category / Metrics	N	Average Baseline Value (SD)	Average Week 12 Value (SD)	Average Change	P Value
Weight (lbs.)	16	182.57 (35.9)	179.30 (33.0)	-3.3	<0.01
Body Mass Index	16	32.83 (6.3)	32.04 (5.9)	-0.8	<0.01
Total Cholesterol (mg/dl)	16	198.38 (36.4)	196.69 (44.2)	-1.7	0.7954
High Density Lipids (mg/dl)	16	54.44 (12.4)	52.25 (12.8)	-2.2	0.0928
Low Density Lipids (mg/dl)	16	114.50 (28.7)	118.63 (38.4)	4.1	0.5290
Triglycerides (mg/dl)	16	155.13 (90.6)	132.81 (73.4)	-22.3	0.0926
Systolic Blood Pressure	16	134.75 (18.8)	124.50 (14.1)	-10.3	0.0763
Diastolic Blood Pressure	16	80.63 (11.3)	73.75 (8.1)	-6.9	<0.05
Depression Scale [CES-D]	16	15.31 (10.2)	11.44 (10.4)	-3.9	0.0914
Hostility Scale [Cook-Medley]	16	7.06 (4.4)	5.25 (3.3)	-1.8	0.0720
Daily Total Fat (grams	8	58.62 (39.1)	44.48 (5.8)	-14.1	0.3394
Daily Saturated Fat (grams)	8	19.77 (19.5)	11.77 (3.4)	-8.0	0.2853
Perceived Stress Scale [PSS]	16	17.00 (7.2)	12.88 (6.5)	-4.1	<0.05
Avg. CCA/Mean IMT	16	0.735 (0.1488)	0.810 (0.1677)	0.075	<0.01
Avg. CCA / Max IMT	16	0.865 (0.1556)	0.928 (0.2046)	0.1	<0.05
Fasting Glucose (mg/dl)	16	107 (28.8)	109 (25.7)	2.4	0.6604

HgbA1c	16	6.3 (0.87)	6.5 (0.77)	0.2	0.3545
Cortisol	16	12.8 (3.83)	16.5 (5.44)	3.7	0.0507
TSH	16	1.71 (1.342)	2.07 (1.674)	0.4	0.2887
Epworth Sleepiness Scale (0 to 24)	16	9 (4.5)	8 (4.2)	-0.9	0.4320
Pittsburgh Sleep Quality Index (0-21)	16	10 (4.8)	8 (4.4)	-2.5	0.0512

Table 4B. Comparison of baseline to Year 1 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	14	180.49 (35.5)	177.30 (32.7)	-3.2	<0.05
Body Mass Index	14	32.49 (6.4)	31.66 (6.0)	-0.8	<0.05
Total Cholesterol (mg/dl)	14	201.07 (37.3)	200.21 (45.8)	-0.9	0.9083
High Density Lipids (mg/dl)	14	54.64 (13.1)	52.14 (13.7)	-2.5	0.0715
Low Density Lipids (mg/dl)	14	116.79 (30.0)	121.57 (40.2)	4.8	0.5252
Triglycerides (mg/dl)	14	157.21 (95.4)	136.71 (76.1)	-20.5	0.1721
Systolic Blood Pressure	14	134.00 (18.4)	125.14 (15.0)	-8.9	0.1451
Diastolic Blood Pressure	14	79.57 (11.3)	72.86 (8.3)	-6.7	0.0838
Depression Scale [CES-D]	14	13.71 (9.7)	11.29 (11.0)	-2.4	0.1056
Hostility Scale [Cook-Medley]	14	6.36 (4.2)	4.79 (3.2)	-1.6	0.3330
Perceived Stress Scale [PSS]	14	16.29 (7.4)	12.71 (6.8)	-3.6	<0.05
Daily Total Fat (grams)	10	61.64 (36.5)	54.64 (25.4)	-7.0	0.4039
Daily Saturated Fat (grams)	10	21.49 (18.2)	15.26 (8.5)	-6.2	0.1507
Avg. CCA/Mean IMT	14	0.745 (0.1557)	0.826 (0.1718)	0.081	<0.01
Avg. CCA / Max IMT	14	0.879 (0.1607)	0.948 (0.2110)	0.1	<0.05
Fasting Glucose (mg/dl)	14	109 (30.6)	111 (27.4)	2.0	0.7535
HgbA1c	14	6.4 (0.91)	6.6 (0.77)	0.2	0.3356
Cortisol	14	12.4 (3.61)	16.9 (5.61)	4.5	<0.05
TSH	14	1.66 (1.419)	2.19 (1.766)	0.5	0.1559
Epworth Sleepiness Scale (0 to 24)	14	8 (4.5)	8 (4.5)	-0.6	0.6020
Pittsburgh Sleep Quality Index (0-21)	14	10 (5.1)	8 (4.6)	-2.7	0.0639

Table 4C. Comparison of baseline to 18 month data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	10	189.23 (36.5)	188.36 (33.3)	-0.9	0.7195
Body Mass Index	10	33.66 (7.0)	33.37 (6.2)	-0.3	0.4956
Total Cholesterol (mg/dl)	10	200.50 (40.4)	211.50 (60.3)	11.0	0.4066
High Density Lipids (mg/dl)	10	53.50 (13.3)	52.00 (13.2)	-1.5	0.3974
Low Density Lipids (mg/dl)	10	113.40 (31.1)	126.60 (43.3)	13.2	0.1605
Triglycerides (mg/dl)	10	180.80 (103.6)	163.20 (115.1)	-17.6	0.3961
Systolic Blood Pressure	10	135.80 (20.2)	137.40 (18.0)	1.6	0.8362
Diastolic Blood Pressure	10	79.40 (12.6)	75.20 (11.8)	-4.2	0.2921
Depression Scale [CES-D]	10	13.20 (9.6)	6.80 (7.1)	-6.4	<0.05
Hostility Scale [Cook-Medley]	10	7.40 (4.4)	5.90 (3.6)	-1.5	0.1604
Perceived Stress Scale [PSS]	10	16.00 (8.2)	8.90 (6.8)	-7.1	<0.01
Daily Total Fat (grams)	10	61.64 (36.5)	37.96 (12.0)	-23.7	0.0766
Daily Saturated Fat (grams)	10	21.49 (18.2)	10.20 (4.2)	-11.3	0.0943
Avg. CCA/Mean IMT	10	0.751 (0.1695)	0.775 (0.1719)	0.024	0.4697
Avg. CCA / Max IMT	10	0.888 (0.1815)	0.919 (0.2275)	0.0	0.4407
Fasting Glucose (mg/dl)	10	110 (34.8)	112 (39.7)	1.8	0.6648
HgbA1c	10	6.4 (0.98)	6.7 (1.52)	0.3	0.3126
Cortisol	10	12.8 (3.83)	13.1 (4.67)	0.3	0.8983
TSH	10	1.64 (1.577)	1.44 (1.243)	-0.2	0.5141
Epworth Sleepiness Scale (0 to 24)	10	8 (4.7)	7 (4.0)	-0.7	0.5496
Pittsburgh Sleep Quality Index (0-21)	10	10 (5.0)	7 (3.4)	-3.1	0.1121

Table 4D. Comparison of baseline to year 2 data for participants in the STEP program

Category / Metrics	N	Average Baseline Value (SD)	Average Year 1 Value (SD)	Average Change	P Value
Weight (lbs.)	10	189.23 (36.5)	184.84 (31.3)	-4.4	0.1403
Body Mass Index	10	33.66 (7.0)	32.58 (5.7)	-1.1	0.1971
Total Cholesterol (mg/dl)	10	200.50 (40.4)	194.60 (53.8)	-5.9	0.5141
High Density Lipids (mg/dl)	10	53.50 (13.3)	48.70 (11.9)	-4.8	<0.001
Low Density Lipids (mg/dl)	10	113.40 (31.1)	114.70 (43.3)	1.3	0.8637
Triglycerides (mg/dl)	10	180.80 (103.6)	162.20 (118.8)	-18.6	0.4207
Systolic Blood Pressure	10	135.80 (20.2)	132.60 (15.3)	-3.2	0.4981
Diastolic Blood Pressure	10	79.40 (12.6)	76.40 (10.6)	-3.0	0.3412
Depression Scale [CES-D]	10	13.20 (9.6)	12.00 (11.9)	-1.2	0.7045
Hostility Scale [Cook-Medley]	10	7.40 (4.4)	6.40 (5.3)	-1.0	0.3765
Perceived Stress Scale [PSS]	10	16.00 (8.2)	13.00 (9.1)	-3.0	0.2401
Daily Total Fat (grams)	10	61.64 (36.5)	56.12 (20.1)	-5.5	0.7037
Daily Saturated Fat (grams)	10	21.49 (18.2)	17.05 (6.0)	-4.4	0.5093
Avg. CCA/Mean IMT	10	0.751 (0.1695)	0.738 (0.1484)	-0.013	0.7605
Avg. CCA / Max IMT	10	0.888 (0.1815)	0.845 (0.1683)	0.0	0.3379
Fasting Glucose (mg/dl)	10	110 (34.8)	111 (30.9)	0.6	0.8474
HgbA1c	10	6.4 (0.98)	6.4 (0.94)	0.0	0.5987
Cortisol	10	12.8 (3.83)	14.7 (4.31)	1.8	0.1363
TSH	10	1.64 (1.577)	2.22 (1.635)	0.6	0.2716
Epworth Sleepiness Scale (0 to 24)	10	8 (4.7)	7 (4.5)	-0.7	0.6380
Pittsburgh Sleep Quality Index (0-21)	10	10 (5.0)	7 (4.3)	-3.1	0.0895

During this year, we received data on the following variables for CRC participants. Analysis will be forthcoming.

NMR lipid panel	296	Insulin	106
CRP	148	Adiponectin	0
Leptin	106	Serum Amyloid	0
Lipoprotein (a)	0	Resistin	106
Vitamin D	69		

Adverse Events:
All adverse events are submitted to and adjudicated by the Windber Medical Center Institutional Review Board and TATRC after review by both the Principal Investigator and Medical Monitor. There was one adverse event during the last quarter, the event was deemed serious, not related and unexpected due to testing that revealed terminal metastasis to the bone and adrenal gland. To date, there have been 5 adverse events, 4 were deemed serious and 1 event was not serious. A serious event is defined as occurring at any dose or intervention level that results in any of the following outcomes: (1) results in death, (2) a threat to life, (3) inpatient hospitalization or prolongation of existing hospitalization, (4) persistent or significant disability or incapacity, (5) causes cancer, (6) is an overdose, or (7) any medical event that requires treatment to prevent one of the medical outcomes listed above. Three of the events were considered serious due to inpatient hospitalizations and one due to poor prognosis related disease progression. No deaths occurred and none of these adverse events were deemed to be study related.

Task #12: Defining the Genetic Basis of Heart Attack and Acute Coronary Syndromes in Military Service Women.
This study will identify genes that affect susceptibility to heart attack in young military service personnel who have had a heart attack before the age of 55. Patients will be selected from the Department of Defense Serum Repository, which has millions of serum samples in storage. Cutting-edge technology will be used to isolate very small amounts of DNA that can be found in serum. More than 1,000,000 variations in the DNA will be tested. The ultimate objective is to identify new genes that increase risk for heart attack at an early age – such genes represent new targets for preventive or therapeutic interventions.

Status:
We have revised the study protocol, which will be initiated as a feasibility study. This modification in the study design will determine the feasibility of isolating and genotyping quality DNA from serum samples in the Department of Defense Serum Repository (DoDSR). For this proof-of-principal study we aim to: (1) assess the quantity and quality of DNA isolated from serum samples obtained from the DoDSR and (2) evaluate the performance of the obtained DNA on Affymetrix 6.0 SNP arrays containing 1.6 million markers. These preliminary studies will determine if we can use DoDSR DNA on high-density genetic marker arrays for future studies.

We continued our research and development work on whole-genome amplification of DNA samples and large-scale genomic research on these samples. The following abstracts were presented at the Association for Molecular Pathology November 2011 meeting. Abstracts were published in the Journal of Molecular Diagnostics.

- Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, November 17-19, Grapevine, TX.

- Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism

arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, November 17-19, Grapevine, TX.

Research and development work continued. Using laboratory samples that should be similar to the repository samples, call rates for all genomic DNA samples were all >97.90% (Table 5). Call rates for DNA isolated from serum were >93.00% and for DNA isolated from heparin plasma were >95.7%. Samples from EDTA tubes that were whole-genome amplified did not perform well (~69-89% call rates). Serum samples from the DoDSR will be compared to these samples in the next period.

Table 5. Call rates on Affymetrix 6.0 arrays for DNA from various sources.

Sample	P/S	CQC	Call Rate
#1 Genomic	N/A	3.04	98.78
#2 Genomic	N/A	2.63	97.9484
#3 Genomic	N/A	2.43	97.9153
#5 Genomic	N/A	2.67	98.0477
#1 Serum Unamplified	Serum	0.72	93.051
#2 Serum Unamplified	Serum	2.32	97.7498
#3 Serum Unamplified	Serum	2.21	98.2793
#5 Serum Unamplified	Serum	2.25	97.85
#1 Serum WGA	Serum	2.19	96.1946
#2 Serum WGA	Serum	-0.05	84.71
#3 Serum WGA	Serum	2.15	96.1284
#5 Serum WGA	Serum	0.72	90.7346
#1 EDTA Unamplified	Plasma	0.47	89.74
#2 EDTA Unamplified	Plasma	3.31	99.01
#3 EDTA Unamplified	Plasma	-0.46	87.7895
#5 EDTA Unamplified	Plasma	0.43	91.4295
#1 EDTA WGA	Plasma	-0.07	77.9616
#2 EDTA WGA	Plasma	0.01	88.88
#3 EDTA WGA	Plasma	-0.03	68.63
#5 EDTA WGA	Plasma	-0.06	73.0311
#1 Heparin Unamplified	Plasma	1.67	95.7313
#2 Heparin Unamplified	Plasma	2.6	97.1873
#3 Heparin Unamplified	Plasma	2.6	98.84
#5 Heparin Unamplified	Plasma	2.41	98.1469
#1 Heparin WGA	Plasma	2.02	94.143
#2 Heparin WGA	Plasma	2.75	98.5109
#3 Heparin WGA	Plasma	2.21	97.3527
#5 Heparin WGA	Plasma	2.61	97.9815

Task #15: Metabolic and Molecular Biology Studies in Surgical Interventions for Morbid Obesity.

The protocol received second level approval from TATRC on June 20, 2012. Since that time, we consented patients to participate in this study and obtained blood and tissue samples. Fourteen lap-band patients were consented to participate in the research study; 32 follow-up blood samples were collected and 708 aliquots were processed and stored as summarized below:

Consented patients	14	
Paxgene tubes for RNA	46	
Adipose tissue (omentum & subcutaneous)		28
Plasma		200
Low-volume plasma	131	
RBCs	157	
NMR lipids	44	
Leptin	44	
Insulin	44	
hsCRP	44	

Key Research Accomplishments

- Dr. Dean Ornish Program for Reversing Heart Disease protocol
    - Subject enrollment over 25 cohorts is complete – 422 participants were enrolled, 339 participants graduated, 83 participants dropped out
    - Age/gender/disease status matched control group established to compare risk factor changes

- Global Profiling of Gene/Protein Expression and Single Nucleotide Polymorphisms Associated with Coronary Heart Disease Reversal
    - Subject enrollment was 374 – 166 participants in the lifestyle change program, 140 subjects serving as the control group, and 68 participants enrolled in the Sub-study
    - One abstract presented at Obesity 2012 Annual Scientific Meeting in San Antonio, TX.
    - Two abstracts presented at the Association for Molecular Pathology (AMP) 2011 Annual Meeting in Dallas, TX
    - Two abstracts accepted to upcoming meetings: American Heart Association Scientific Sessions 2012 in Los Angeles, CA and the American Society of Human Genetics Meetings in San Francisco, CA.
    - Participation in the Program reduces levels of important biochemical risk factors for CAD, such as CRP and MIF (manuscripts in progress).
    - Changes in gene expression mirror changes in many CVD risk factors – dramatic decrease during the first 12 weeks, then regression toward baseline from week 13 to 52.
    - Most cholesterol and lipid homeostasis genes show a continual decrease in expression throughout the program similar to body weight.
    - Medication use clearly does not affect gene expression, thus expression changes may be attributed to the lifestyle change program
    - Genetic variation influences risk factor response
        --Several SNPs show evidence of an influence on triglyceride response

- The Cardiovascular Risk Clinic (CRC)
    - Subject enrollment is 132; 114 participants remain active

- The Stress Therapy Empowering Prevention (STEP) program
    - Two abstracts presented at the National Consortium of Breast Centers Interdisciplinary Breast Center Conference in Las Vegas

Reportable Outcomes

Published Manuscripts/Abstracts:

Burke A, Ellsworth DL, Vernalis MN. Stress Therapy Empowers Prevention (STEP): A Healthy-Lifestyle Program for Breast Cancer Patients. J Oncol Navig Surviv 2012;3(1):8-14.

Voeghtly LM, Neatrour DM, Decewicz DJ, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. Improvement in cardiometabolic risk factors during an intensive cardiovascular lifestyle intervention. Nutr Metab Cardiovasc Dis 2012 (in press).

Ellsworth RE, Valente AL, Shriver CD, Bittman B, Ellsworth DL. Impact of lifestyle factors on quality of life and prognosis in breast cancer survivors in the United States. Expert Rev Pharmacoecon Outcomes Res 2012; in press.

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. (in preparation).

Ellsworth DL, Croft DT Jr, Weyandt J, Field LA, Patney HL, Burke A, Haberkorn MJ, McDyer FA, Jellema GL, van Laar R, Mamula KA, Vernalis MN. Intensive cardiovascular risk reduction induces sustainable changes in peripheral blood gene expression. (in preparation).

Presentations (Oral & Poster):

Voeghtly L, Croft DT Jr, Deyarmin B, Vernalis MN, Shriver CD, Ellsworth DL. Utility of whole genome amplification for assessing copy number variation with high density SNP arrays from formalin-fixed paraffin embedded tissue. Association for Molecular Pathology (AMP) 2011 Annual Meeting, November 17-19, Grapevine, TX.

Croft DT Jr, Voeghtly L, Patney HL, Shriver CD, Vernalis MN, Ellsworth DL. Performance of whole-genome amplified DNA isolated from serum and plasma for estimating copy number variation with high density single nucleotide polymorphism arrays. Association for Molecular Pathology (AMP) 2011 Annual Meeting, November 17-19, Grapevine, TX.

Ellsworth DL, Croft DT Jr, Burke A, Haberkorn MJ, Patney HL, Mamula KA, Vernalis MN. The importance of weight loss for effecting molecular change during intensive cardiovascular risk reduction. Obesity 2012: 30th Annual Scientific Meeting, September 20-24, 2012, San Antonio, TX.

Miller EJ, Mamula KA, Leng L, Piecychna M, Vernalis MN, Bucala R, Ellsworth DL. Cardiovascular disease risk factor modification decreases HS-CRP and Macrophage Migration Inhibitory Factor (MIF): Influence of gender. American Heart Association Scientific Sessions 2012, November 3-7, 2012, Los Angeles, CA. (accepted)

Decewicz A, Hicks M, Mamula KA, Burke A, Haberkorn MJ, Patney HL, Vernalis MN, Ellsworth DL. SNPs associated with plasma triglyceride levels influence response during intensive cardiovascular risk reduction. American Society of Human Genetics, November 6-10, 2012, San Francisco, CA. (accepted)

Conclusions

Unhealthy lifestyle behaviors are linked to the development of CHD, as well as other chronic diseases. Projections based on combined CVD risk factor impact suggest that favorable lifestyle habits could nearly eliminate the development of CHD and substantially decrease CHD morbidity and mortality. We have demonstrated that comprehensive lifestyle interventions are remarkably efficacious in reducing CVD risk factors and, in many cases, are comparable to pharmacological interventions. We also have shown that molecular change occurs during lifestyle modification, but this change may be transient and may be dependent on maintaining a healthy lifestyle. Future research endeavors from this project will provide new information regarding strategies to improve adoption of healthy lifestyle behaviors, the impact of lifestyle interventions on CVD risk, and the biologic mechanisms through which lifestyle changes exert their influence. Through this research, the DOD has a unique opportunity to identify and address adverse lifestyle behaviors and CVD risk factors early and make cardiovascular health a part of the military culture. A commitment to CV health could prevent cardiac events, reduce the need for costly procedures and hospitalization, improve quality of life and protect the investment of highly trained military personnel.